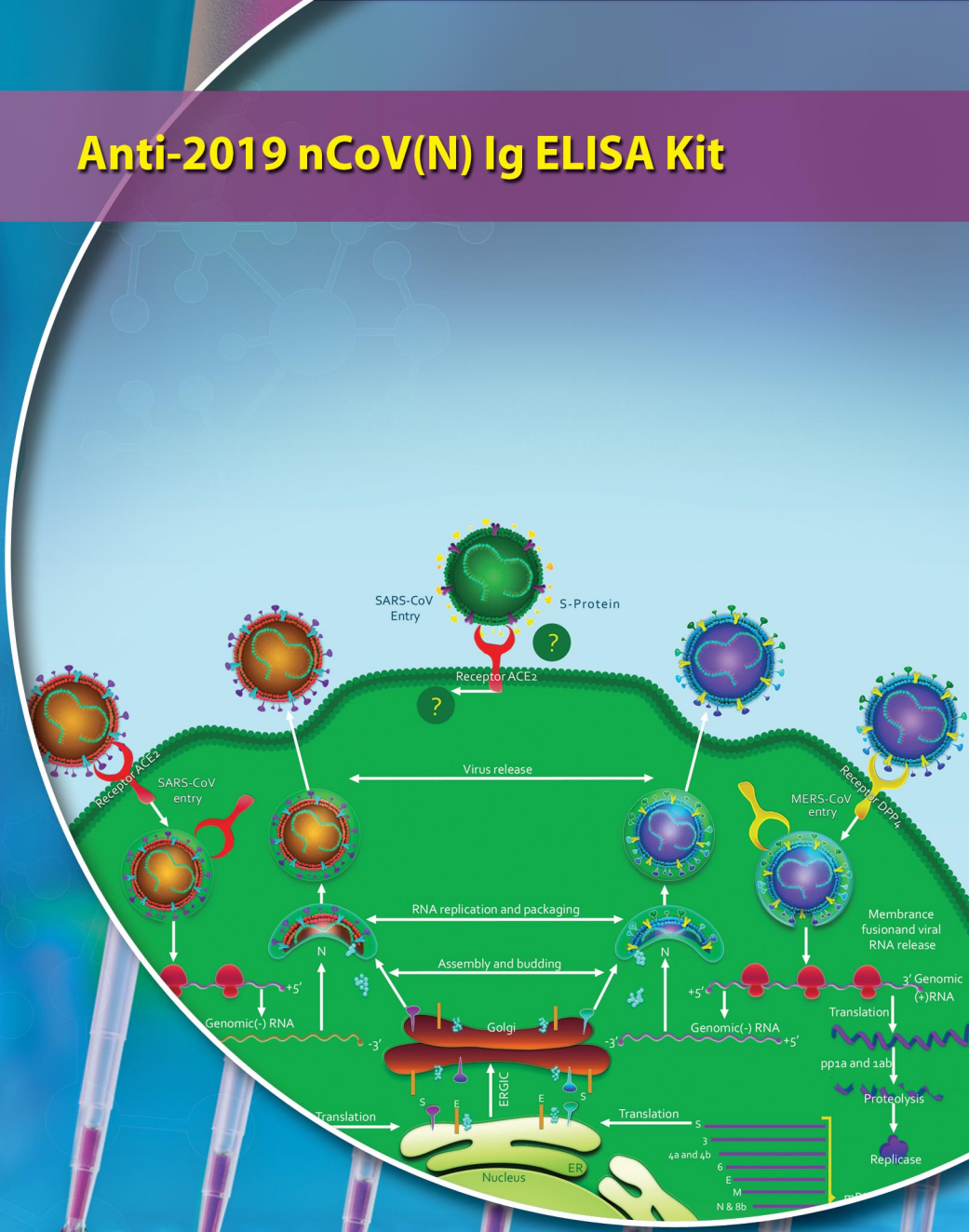
Anti-2019 nCoV(N) Ig ELISA Kit



Cat.No: 90-7007

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Anti-2019 nCoV(N) Ig ELISA Kit

Catalog No: 90-7007
1 × 96 well Format (96 tests)
Reactivity: COVID-19, SARS-CoV-2
Detection Range: 62.5-4000pg/ml
Sensitivity: <37.5pg/ml

This immunoassay kit allows for the qualitative determination of 2019-nCoV(N)-Ig antibody in serum, plasma and saliva and nasal fluid.

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I. PRINCIPLE OF THE ASSAY

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Recombinant 2019-nCoV Nucleocapsid protein (antigen) was pre-coated onto 96-well plates. The Controls, test samples and Biotin- labeled antigen were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin Conjugate was added and unbound conjugates were washed away with wash buffer. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

II. OVERVIEW (Sequence of the Nucleocapsid Protein (antigen))

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWF
TALTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPR
WYFYYLGTGPEAGLPYGANKDGIIWVATEGALNTPKDHIGTRNPANNAAIVLQ
LPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGTSPARMAGNGG
DAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAY
NVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIG
MEVTPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKK
KADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQSMSSADSTQA

III. ADVANTAGES

Multiple samples can be analyzed in a low volume, high-throughput format. Full analysis can be completed in 2 hours.

IV. REQUIRED INSTRUMENTS AND REAGENTS

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator (CO2 incubator for cell culture is not recommended.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water.

V. SAMPLE COLLECTION AND STORAGE

1. Serum

Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

2. Plasma

Collect plasma using (EDTA-Na2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

3. Saliva & Nasal fluid

Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C (assay ≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

VI. KIT COMPONENTS

Item	Specifications (96T)	Storage
Coated assay plate	1vial	2-8°C
Negative Control (Ready-to-use)	1vial	2-8°C
Positive Control (Ready-to-use)	1vial	2-8°C
Sample Dilution Buffer	1vial	2-8°C
Biotin-conjugated Nucleocapsid (Concentrated)	1vial	2-8°C in dark
Antigen Dilution Buffer	1vial	2-8°C
HRP-Streptavidin Conjugate (SABC)	1vial	2-8°C in dark
SABC Dilution Buffer	1vial	2-8°C
Wash Buffer (25 x concentrate)	1vial	2-8°C
TMB Substrate	1vial	2-8°C in dark
Stop solution	1vial	2-8°C
Plate Sealer	5 pieces	
Product Manual	1 copy	

VII. PRECAUTIONS FOR USE

1. When using different Elisa kits, labeling is required to avoid mixed components and failed assay.

2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace (e.g. E002 lyophilized standard).

3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.

4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.

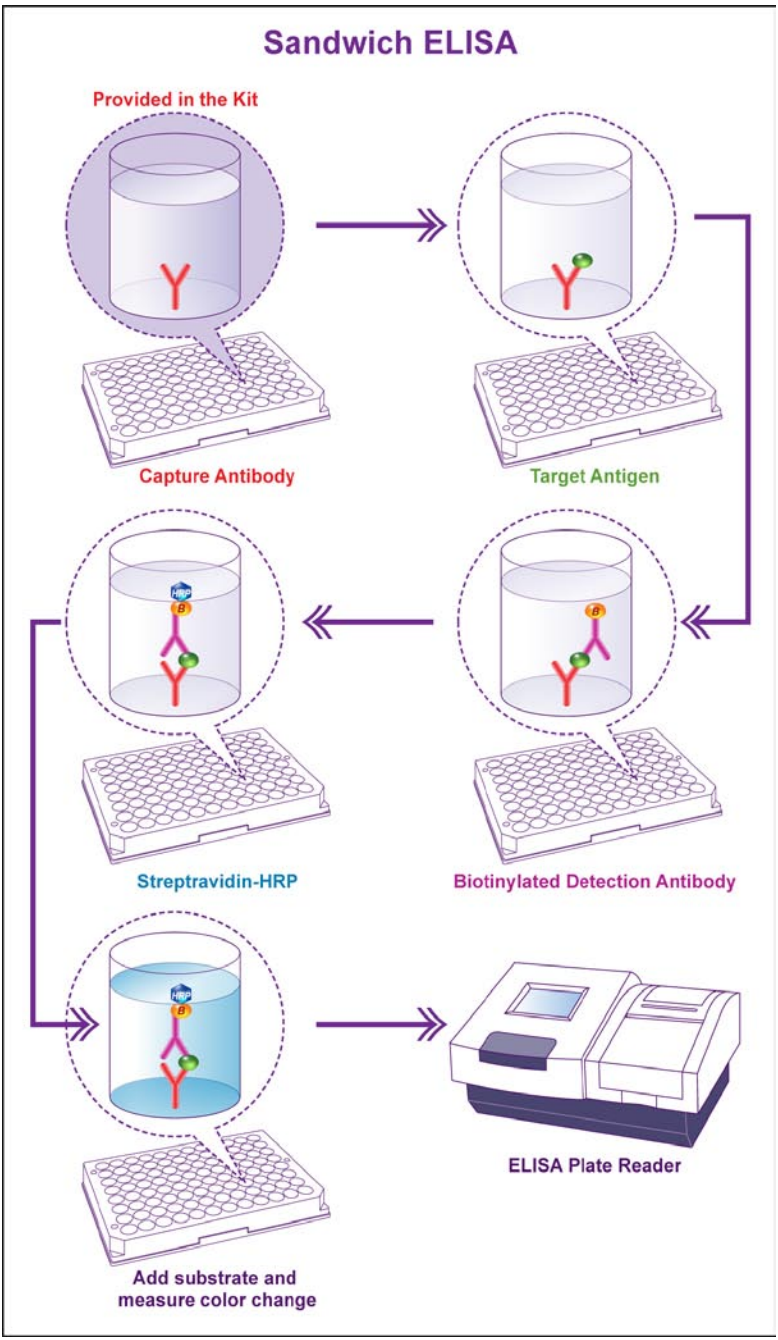
5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.

6. Before confirmation, reagents from other batches or sources should not be used in this kit.

7. Don't reuse tips and tubes to avoid cross contamination.

8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.

9. Please wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples, please follow regulations on safety protection of biological laboratory.


Sandwich ELISA
Provided in the Kit
Capture Antibody
Target Antigen
Streptravidin-HRP
Biotinylated Detection Antibody
ELISA Plate Reader
Add substrate and
measure color change

VIII. REAGENT PREPARATION AND STORAGE

Included buffers and reagents are optimized for use with this kit. Substitution with other reagents is not recommended and may not give optimal results.

Bring all reagents and samples to room temperature for 20 minutes before use.

1. **Wash Buffer:**

   If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

   Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

2. **Preparation of Biotin-labeled Antigen Working Solution:**

   Prepare it within 30minutes before experiment.

   a. Calculate required total volume of the working solution: 50 µL/well × quantity of wells. (Allow 55-60 µL more than the total volume).

   b. Dilute the Biotin-labeled Antigen with Antigen Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 µL Biotin- labeled Antigen into 99µL Antigen Dilution Buffer).

3. **Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:**

   Prepare it within 30 minutes before experiment.

   a. Calculate required total volume of the working solution: 50 µL /well × quantity of wells (Allow 55-60 µL more than the total volume).

   b. Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly (i.e. Add 1 µL of SABC into 99 µL of SABC Dilution Buffer).

IX. ASSAY PROCEDURE

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Bring all reagents to room temperature before use.
2. Label the sample wells, 2 Negative Controls, 2 Positive Controls and 1 blank well. Adding sample and control (blank) wells!
3. Add 40 μL sample dilution buffer to each sample well. Add 50 μL sample dilution buffer to blank well.
4. Add 10 μL sample to each sample well. Add 50 μL Negative Controls and Positive Controls to set Controls well and gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 30 minutes.
5. Remove the cover, and wash plate 2 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 minutes.
6. Add 50 μL Biotin-labeled Antigen to each well.
7. Seal the plate with cover and incubate at 37°C for 60 min.
8. Remove the cover, and wash plate 3 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 minutes.
9. Add 50 μL of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.
10. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 minutes.
11. Add 50 μL of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark within 10-15 minutes. And the shades of obvious blue can be seen in the Positive Controls. Blank well wells show no obvious color.
12. Add 50 μl of stop solution into each well and mix thoroughly. The color changes into yellow immediately.
13. Read O.D. absorbance at 450 nm in a micro-plate reader immediately after adding the stop solution (Use the blank well to set zero).

X. DATA ANALYSIS

Cutoff Value =NCx× 2.1

NCx: Mean Absorbance of Negative Control (when NCx<0.05, Calculate as 0.05).

PCx: Mean Absorbance of Positive Control

1. Sample with absorbance values < Cutoff Value are considered negative.

Sample with absorbance value ≥ Cutoff Value are considered positive.

2. PCx≤0.5, the test is regarded as invalid, should be tested again.

Sample test data (for reference only)

Samples came from rehabilitation clients (1-2 months after recovery) of mobile cabin hospital. The plasma samples were diluted 1:5. TMB Color development time was 15 minutes at 37°C. NCx=0.109

Rehabilitation clients (OD450)				**Healthy volunteers (OD450)**			
1#	1.850	9#	1.876	1#	0.101	9#	0.112
3#	1.371	10#	1.556	2#	0.094	10#	0.095
3#	1.962	11#	2.010	3#	0.077	11#	0.088
4#	1.984	12#	1.458	4#	0.09	12#	0.099
5#	1.985	13#	1.957	5#	0.143	13#	0.134
6#	1.885	14#	2.144	6#	0.128	14#	0.120
7#	2.046	15#	2.202	7#	0.112	15#	0.122
8#	1.295	16#	1.891	8#	0.096	blank	0.139

XI. TROUBLE SHOOTING

Problem	Probable Cause	Suggestion
No signal	Forgot to add all components.	Prepare check list and add the components in the correct order.
Low signal	Not enough lysates per well.	Check the protein concentration. Add more lysates.
High background	Washing is not sufficient.	Wash plates thoroughly after incubation with Streptavidin-HRP secondary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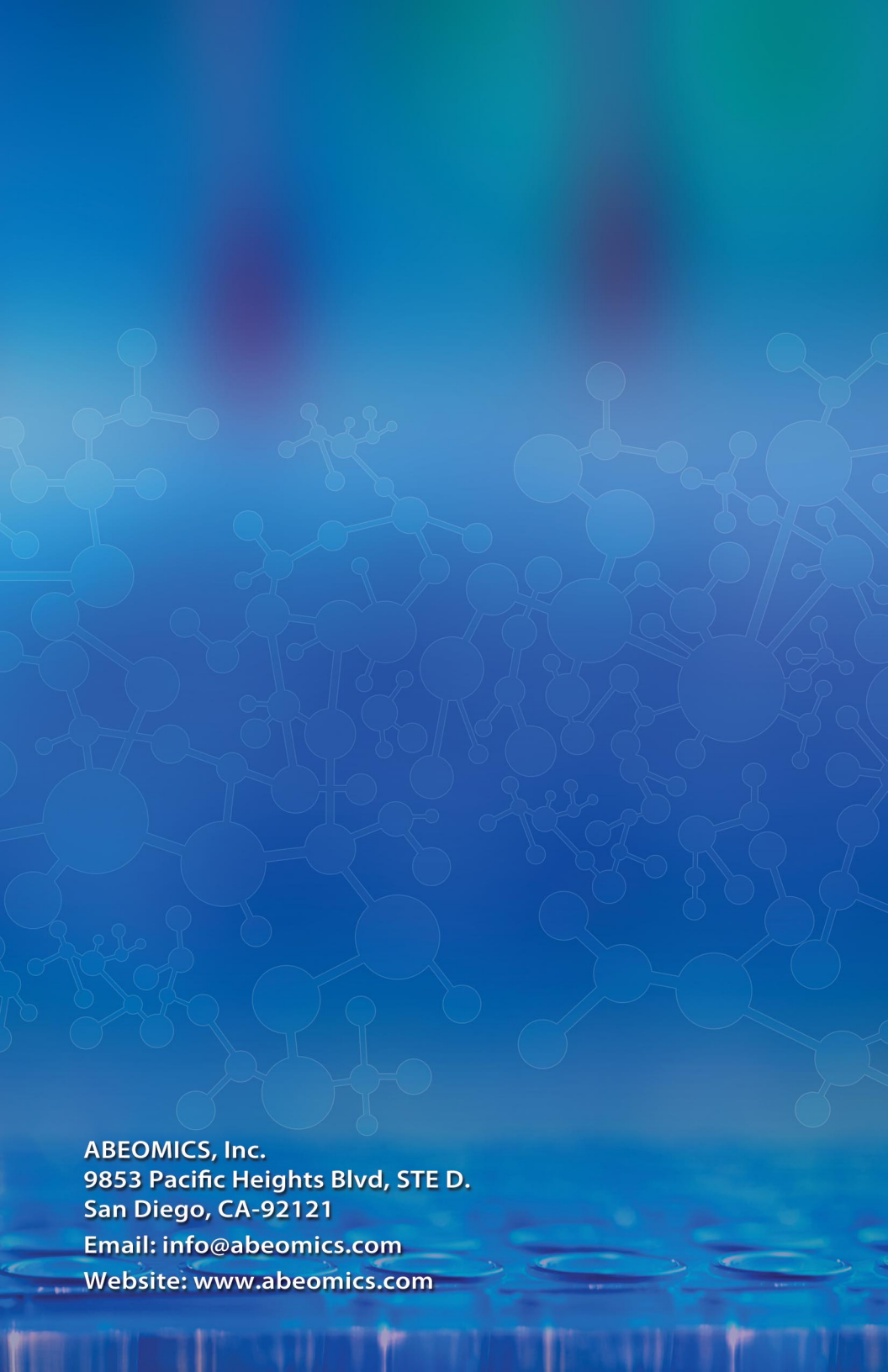
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